METHOD FOR DETERMINING THE RESPONSE TO CANCER THERAPY

This application is a continuation of U.S. Provisional Patent Application Nos. 60/176,514 and 60/176,515, each filed on January 12, 2000, the disclosure of each of which is explicitly incorporated by reference herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to methods for determining or predicting response to cancer therapy in an individual. In particular, the invention relates to methods for using image analysis to assess the efficacy of chemotherapeutic and chemopreventative agents in a human in need of treatment with such agents by detecting expression levels of biological makers associated with senescence, apoptosis or terminal differentiation. More specifically, the invention provides methods where the amount of the senescence, apoptosis or terminal differentiation marker is quantitated in tissue and cell samples removed from an individual before and after exposure to the chemotherapeutic or chemopreventative agent.

2. Background of the Invention

A primary goal of cancer therapy is to selectively kill or inhibit the uncontrolled growth of malignant cells while not adversely affecting normal cells. Traditional chemotherapeutic agents are highly cytotoxic agents that preferably have greater affinity for malignant cells than for normal cells, or at least preferentially affect malignant cells based on their high rate of metabolic activity. However, these agents often harm normal cells.

In the use of anticancer drugs, monoclonal antibodies, or chemopreventive agents, growth arrest, terminal differentiation and cell death of the cancerous or precancerous cells is intended (Mendelsohn, 1990, *Semin. Cancer Biol.* 1:339-44; Hancock *et al.*, 1991, *Cancer Res.* 51:4575-80; Arteaga *et al.*, 1994, *Cancer Res.*, 54:3758-65; Pietras *et al.*, 1994, *Oncogene* 9:1829-38; Bacus *et al.*, 1997, *Anal. Quant. Cytol. Histol.* 19:316-28; Bacus *et al.*, 1999, *Breast J.*; Baselga *et al.*, 1999, Proceedings of AACR NCI EORTC International Conference, Abstract 98; Cobleigh *et al.*, 1999, *J. Clin. Oncol.*17:2639-48; DiGiovanna, 1999, *PPO Updates: Princ.*

Practice Oncol. 13:1-9; Hortobagyi, 1999, *J. Clin. Oncol.* 17:25-29; Shak, 1999, *Semin. Oncol.* 26:71-77; Sliwkowski *et al.*, 1999, *Semin. Oncol.* 26:60-70; Vincent *et al.*, 2000, *Cancer Chemother. Pharmacol.* 45: 231-38). Drug-induced growth arrest or cell death is often characterized by morphological and biochemical changes associated with programmed cell death or terminal differentiation (as opposed to mitotic cell death).

Although chemotherapeutic drugs can be administered at doses high enough to bring about cell death, such doses typically produce deleterious effects on normal as well as tumor cells. Differentiating agents, and lower doses of chemotherapeutic drugs and agents frequently results in growth arrest rather than cell death; such arrest can be followed by apoptosis and sell death, or continued proliferation once the chemotherapeutic drugs are withdrawn. Administration of cytotoxic and chemotherapeutic drugs or ionizing radiation may also induce transient growth arrest, a state which depends largely on the function of p53 and a p53-regulated cyclin-dependent kinase inhibitors (such as p16, p27, and p19) or growth inhibitors (such as TGF-β, IL-4, and IL-6). Upon removal of the chemotherapeutic drug, cells subjected to the drug treatment will eventually resume division and either continue to proliferate or die. Some drug-treated tumor cells undergo prolonged growth arrest and fail to resume cell division upon release from the drug.

In normal cells, terminal proliferation arrest may result from terminal differentiation or replicative senescence. Senescence is a physiological process that limits the proliferative span of normal cells. A commonly-used marker of senescence in human cells is expression of senescence-associated β-galactosidase (SA-β-Gal). This protein has been shown to correlate with senescence in aging cell cultures *in vitro* and with cells *in vivo*. Terminal proliferation arrest in normal cells can be rapidly induced by treatment with DNA-damaging drugs or γ-irradiation and is accompanied by the morphological features of senescence and the induction of SA-β-Gal. Accelerated senescence is likely to be a protective response of cells to carcinogenic impact. Similar to other damage responses of normal cells – such as quiescence and apoptosis – senescence-like terminal proliferation arrest involves the function of proteins such as wild-type p53 and the up-regulation of such proteins as $p21^{WAF1}$, p16, p19, and p27 (Kwok and Sutherland, 1989, *J. Natl. Cancer Inst.* 81:1020-24; Kwok and Sutherland, 1991, *Int. J. Cancer* 49:73-76; Kastan *et al.*, 1991, *Cancer Res.* 51:6304-11; Lane, 1992, *Nature* 358:15-16; Kuerbitz *et al.*, 1992, *Proc. Natl. Acad. Sci. U.S.A.* 89:7491-95; Gu *et al.*, 1993, *Nature* 366:707-10; Halevy *et*

al., 1995, *Science* 267:1018-21; Sherr and Roberts, 1995, *Genes Dev.* 9:1149-63; Luo *et al.*, 1995, *Nature* 375:159-61; Dimri *et al.*, 1995, *Proc. Natl. Acad. Sci. U.S.A.* 92:9363-67; Bacus *et al.*, 1996, *Oncogene* 12:2535-47; Liu *et al.*, 1996, *Cancer Res.* 56:31-35; Wang *et al.*, 1998, *Oncogene* 17:1923-30; Chang *et al.*, 1999, *Oncogene* 18:4808-18; Hong *et al.*, 1999, *Cancer Res.* 59:2847-52; Sionov and Haupt, 1999, *Oncogene* 18:6145-57; Wouters *et al.*, 1999, *Oncogene* 18:6540-45).

Tumor cells appear to have retained the ability to enter senescence and terminal proliferation arrest. Treatment of tumor cells with different classes of agents that affect cell differentiation, and anticancer agents, readily induces morphological, enzymatic, and other changes characteristic of senescence (such as the up-regulation of p53, p21, p27, p16, TGF-β, IL-4, IL-6, and SA-β-Gal). This senescence-like phenotype (SLP) distinguishes cells that will become stably growth-arrested from cells that will recover from drug exposure and continue to proliferate. Thus, the induction of senescence-like terminal proliferation arrest provides an important determinant of treatment response in tumor cells.

Apoptosis is generally regarded as an active suicidal response to various physiological or pathological stimuli. Recent studies have shown that a variety of DNA-damaging agents, including X-ray irradiation and several chemotherapeutic drugs (*e.g.*, alkylating agents and topoisomerase II inhibitors) cause necrosis or initiate pathways leading to apoptosis. The exact mechanism by which apoptosis is induced by these agents is not yet known. However, expression of the suppressor gene p53 has been implicated in this process (Kwok and Sutherland, 1989, *J. Natl. Cancer Inst.* 81:1020-24; Kwok and Sutherland, 1991, *Int. J. Cancer* 49:73-76; Lane, 1992, *Nature* 358:15-16; Kuerbitz *et al.*, 1992, *Proc. Natl. Acad. Sci. U.S.A.* 89:7491-95; Luo *et al.*, 1995, *Nature* 375:159-61; Liu *et al.*, 1996, *Cancer Res.* 56:31-35; Mellinghoff and Sawyers, 2000, *PPO Updates* 14:1-11). In addition, the up-regulation of caspases (*e.g.*, caspase 9 or caspase 3) or their chaperone molecules (*e.g.*, heat shock protein 60) has been associated with apoptosis.

In cells undergoing apoptosis, DNA-damaging stimuli can result in an elevation of intracellular p53 protein levels. Increased levels of wild-type p53, in turn, inhibit the cell cycle at G_1, thus permitting the damaged cell to undergo DNA repair. However, if the damaged cell is unable to undergo DNA repair, p53 can trigger programmed cell death. It is this ability to trigger

programmed cell death that contributes to the induction of tumor cell death following exposure to chemotherapeutic agents.

Increased levels of p53 can also lead to the activation of a number of genes that contain wild-type p53-binding sequences, including the MDM-2 oncogene, Bax, and the WAF1/CIP1 gene. The WAF1/CIP1 gene encodes a protein having a M_r of 21,000 that associates with cyclin-Cdk complexes and is capable of inhibiting kinase activity associated with these complexes. A major target of WAF (p21 or CIP) inhibition is the cyclin E-Cdk2 kinase complex whose activity is required for G_0 to S phase cell cycle progression. The WAF1/CIP1 gene is transcriptionally activated in response to DNA-damaging agents that trigger G_1 arrest or apoptosis in cells with wild-type p53 but not in tumor cells harboring deletions or mutations of the p53 gene. However, WAF1/CIP1 has also been shown to be up-regulated in cells undergoing differentiation or cell cycle arrest by a p53-independent mechanism.

Thus, there are a variety of cellular markers of senescence, apoptosis and terminal proliferative arrest that can be used to detect the effects of chemotherapeutic and chemopreventative drugs and agents. These markers can be used to assess the success or failure of any particular chemotherapeutic or chemopreventative drug or agent, or combination thereof, to affect an anticancer effect on tumor cells *in vivo*.

In contrast to traditional anticancer methods, where chemotherapeutic drug treatment is undertaken as an adjunct to and after surgical intervention, neoadjuvant (or primary) chemotherapy consists of administering drugs as an initial treatment in cancer patients. One advantage of such an approach is that, in primary tumors of more than 3 cm this approach permits the use of conservative surgical procedures (as opposed to, *e.g.*, mastectomy in breast cancer patients) in the majority of patients. Another advantage is that for many cancers, a partial and/or complete response is achieved in about two-thirds of all cases. Finally, since the majority of patients are responsive after two to three cycles of chemotherapeutic treatment, it is possible to monitor the *in vivo* efficacy of the chemotherapeutic regimen employed, which is important for a timely identification of those cancers which are non-responsive to chemotherapeutic treatment. Timely identification of non-responsive tumors, in turn, allows the clinician to limit the cancer patient's exposure to unnecessary side-effects and institute alternative treatments.

The efficacy of chemotherapeutic agents in treating particular cancers is unpredictable. In view of this unpredictability, it has not been possible to determine, prior to starting therapy,

whether one or more selected agents would be active as anti-tumor agents or to render an accurate prognosis of course of treatment in an individual patient. It would be very desirable to be able to determine the efficacy of a proposed therapeutic agent (or combination of agents) in an individual patient. There is a need in the art for a method of assessing the efficacy of chemotherapeutic programs that is both time- and cost-effective and minimally traumatic to cancer patients.

SUMMARY OF THE INVENTION

The present invention provides methods for determining the response to cancer therapy in an individual. The invention specifically provides methods for assessing efficacy of chemotherapeutic and chemopreventative agents in a human in need of treatment with such agents by detecting expression levels of biological makers associated with senescence, apoptosis or terminal differentiation. In the inventive methods, the amount of one or a plurality of senescence, apoptosis or terminal differentiation markers is quantitated in tissue and cell samples removed from an individual before and after exposure to the chemotherapeutic or chemopreventative agent. In preferred embodiments, the amount of said marker is determined using image analysis of immunohistochemically-stained tissue or cell samples obtained from a patient tumor.

In one embodiment of the inventive methods, response to a chemotherapeutic or chemopreventive agent in an individual is determined by collecting a first tissue or cell sample from the individual before exposing the individual to the chemotherapeutic or chemopreventive agent, collecting a second tissue or cell sample from the individual after exposing the individual to the chemotherapeutic or chemopreventive agent, immunohistochemically staining the first and second tissue or cell samples using a detectably-labeled antibody directed against a biological marker associated with senescence, apoptosis or terminal differentiation, determining the amount of the marker in the first and second tissue or cell samples, and determining whether expression of the biological marker associated with senescence, apoptosis or terminal differentiation was increased following exposure to the chemotherapeutic or chemopreventive agent. In a preferred embodiment, the detectable label is a chromagen or a fluorophore.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

Figures 1A-1B show the results of analysis of MCF7 cells that were not treated (A) or treated (B) with doxorubicin, and then stained for SA-β-Gal activity;

Figures 2A-2C show the results of flow cytometric analysis of MCF7 cells untreated (A) or treated (B) with doxorubicin, or treated with Taxol (C);

Figures 3A-3C illustrate the results of an analysis of a tumor sample removed from an individual following treatment with Herceptin™ (an antibody to the oncogene protein HER-2/*neu*). Tumor tissue was stained with either Hemotoxin and Eosin (A), SA-β-Gal (24 hours after treatment with Herceptin™; C), or SA-β-Gal 14 days after treatment with Herceptin™.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The methods of the present invention are useful for determining the response to cancer therapy in an individual. Specifically, the present invention provides methods for assessing the efficacy of treating an individual in need of such treatment with a chemotherapeutic or chemopreventive agent, in which the level of expression of a biological marker associated with senescence, apoptosis or terminal differentiation is quantitated in immunohistochemically-stained tissue or cell samples removed from the individual before and after exposure to the chemotherapeutic or chemopreventive agent. In preferred embodiments, expression levels are quantitated using computer-aided image analysis system.

In one embodiment of the methods of this invention, response to a chemotherapeutic or chemopreventive agent in an individual is determined by collecting a first tissue or cell sample from the individual before exposing the individual to the chemotherapeutic or chemopreventive agent, collecting a second tissue or cell sample from the individual after exposing the individual to the chemotherapeutic or chemopreventive agent, immunohistochemically staining the first and

second tissue or cell samples using a detectably-labeled antibody directed against a biological marker associated with senescence, apoptosis or terminal differentiation, determining amount of expression of one or a plurality of biological markers associated with senescence, apoptosis or terminal differentiation in the first and second tissue or cell samples, and determining whether expression of the biological marker(s) associated with senescence, apoptosis or terminal differentiation was increased following exposure to the chemotherapeutic or chemopreventive agent.

In preferred embodiments, biological markers whose expression is induced or increases in cellular senescence, apoptosis and terminal differentiation may include but are not limited to p21, p27, p16, TGF-β, IL-4, IL-6, and SA-β-Gal, generally and collectively known as the senescence-like phenotype (SLP). This senescence-like phenotype (SLP) can be used to distinguish cells with restricted proliferative potential from those that continue to proliferate after drug exposure, suggesting that senescence-like terminal proliferation arrest is an important determinant of treatment response in human cancer.

In cells treated with cytotoxic drugs, SLP induction and cell death appear to be concurrent and independent responses. Thus, both SA-β-Gal$^+$ cells and SA-β-Gal$^-$ cells have a similar probability of undergoing mitotic death during drug treatment or within days following release from the drug. Once the rapid process of cell death is completed, however, SLP can be used to distinguish growth-retarded and non-clonogenic cells from recovering and proliferating cells. The overall outcome of treatment with chemopreventive or chemotherapeutic drugs is therefore determined by a combination of factors responsible for the induction of cell death (mitotic cell death or apoptosis) and senescence-like terminal proliferation arrest.

Exposure to moderate doses of doxorubicin induces SLP. However, when patients are treated with other agents, such as Taxol or Taxoter, apoptosis and up-regulation of p53 and p21 become more evident. The same is true when patients are treated with Herceptin™, a monoclonal antibody to the oncogenic receptor HER-2/*neu*. Thus, SLP induction may be the primary determinant of treatment outcome for cytostatic differentiating agents such as retinoids, and other chemopreventive agents. In addition, image analysis may be used to examine therapeutic response by quantitating the expression of proteins that are involved in activated pathways (*e.g.*, phosphorylated MAP kinase and Akt) in tissues before and after treatment with kinase inhibitors.

Analysis of SLP markers such as β-Gal, p53, and p21 in clinical cancer may provide an important diagnostic approach for monitoring tumor response to different forms of therapy and can be done by calibrated staining for various factors associated with senescence and quantified by microscope based image analysis.

In a specific embodiment of the methods of the present invention, cells are removed from an individual before and after treatment with a putative anti-cancer agent and the cells are fixed using, *e.g.*, paraformaldehyde. Cell samples are then treated with an organic solvent, such as acetone, formalin, or methanol, to render the cells permeable for immunohistological staining. Methods of fixation are known to those of skill in the art.

Where the presence and distribution of p53, p21, p16, p27, or p21-WAF1 protein are determined, the proteins are identified using suitable antibody systems such as sandwich systems using a primary antibody that specifically binds to the protein of interest and a secondary antibody that binds to the primary antibody. The antibody system can then be visualized using suitable stains (Bacus *et al.*, 1999, *Breast J.*). Generally, antibodies and other molecules that specifically bind to these target proteins are known in the art, and the methods of the present invention can be practiced using any antibody or other molecule or collection of molecules that specifically binds to any of said biological markers of senescence, apoptosis or terminal differentiation.

Exemplary primary antibodies that bind to p53, p21, and p16 include the monoclonal antibody Ab-2 (Oncogene Sciences, Cambridge, MA). Similarly, exemplary antibodies that bind to p21 protein include the monoclonal antibody Ab-1 (Oncogene Sciences). An exemplary secondary antibody that may be used with either of these primary antibodies is rabbit anti-mouse IgG (Jackson Labs, West Grove, PA). After exposure to antibodies, an enzyme complex such as ABC complex (Vector Labs, Burlington, CA) is added to complete the antibody sandwich system and render it amenable to staining and counterstaining. Image analysis techniques are used to complete the quantitation.

The preferred embodiment of the method of the present invention and its advantages over previously investigated methods for quantitating the response to cancer therapy are best understood by referring to Figures 1-3 and Examples 1-6. The Examples, which follow, are

illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

β-Galactosidase (β -Gal) Staining

Cells were washed in PBS, fixed for 3-5 minutes at room temperature in 2% formaldehyde and 0.2% glutaraldehyde (or in 3% formaldehyde alone), washed, and incubated at 37°C in fresh senescence-associated β-Gal (SA-β-Gal) staining solution (1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal), 20 mg/ml dimethylformamide; 40 mM citric acid/ sodium phosphate, pH 6.0; 5 mM potassium ferrocyanide; 5 mM potassium ferricyanide; 150 mM NaCl; 2 mM $MgCl_2$. Staining was evident following 2-4 hours of incubation and maximal staining was reached after in 12-16 hours. To detect lysosomal β-Gal, the staining solution was prepared with citric acid/sodium phosphate at pH 4.0 (Baselga *et al.*, 1999, Proceedings of AACR NCI EORTC International Conference, Abstract 98).

EXAMPLE 2

Quantitation of Protein Expression by Computer-Aided Image Analysis

The expression of proteins associated with senescence or apoptosis are quantitated by image analysis using a suitable primary antibody (such as Ab-2 for p53, or Ab-1 for WAF1, HSP60, or caspase 3), secondary antibody (such as rabbit anti-mouse IgG), and tertiary ABC complex.

Tertiary ABC complexes are visualized by treating the sections with diaminobenizidine (DAB) stain under appropriate conditions. In a second step, the tissue is counterstained with another optical enhancement factor, preferably ethyl green. The resulting preparation has green nuclei with varying degrees of brown diaminobenzidine (DAB) precipitate localized to the nuclei (and indicating the level of expression of p53, p21, p16, WAF1, HSP60, or caspase-3 protein). Although a staining technique using peroxidase and ethyl green is exemplary, other stains and optical enhancement factors are also suitable (such as alkaline phosphatase coupled with a specific chromagen such as Fast Red or Fast Green). Spectral studies have shown that the ethyl green stain offers good spectral separation from the DAB precipitate of the immunoperoxidase technique such that different features of the image can be readily separated by filtering at two

different wavelengths. This allows the image to be digitized into two separate images, one in which all the cell nuclei are optically enhanced (ethyl green or Fast Green) and one in which only those tissue areas with receptor staining (DAB) are optically enhanced. In a preferred embodiment, the images can be separated by a 600 nanometer (red) filter to produce an image of the counterstained area and a 500 nanometer (green) filter to produce an image of the tissue areas staining with the DAB precipitate.

To further differentiate those areas, an interactive threshold setting technique can be used in which an operator visualizing the images can set a boundary on the areas under consideration. When the boundaries are set, the images are formed by eliminating all parts of the image that are below the threshold in optical density. A threshold is set for the first image, and a second threshold is set for the second image.

The image processing method then consists of first forming the mask image of the tissues under consideration with the red filter. This mask image is stored and another image for expressed protein quantification is then acquired by using the green filtered version of the same image. Using the filters in combination allows for the optical enhancement of those areas of the tissue mask where tissue components are stained with DAB (which are darkened) and those tissue components having only the green counterstain (which are lightened). An image analysis can then be performed using only those areas of the image that are stained and which are within the mask.

The amount of target protein is then quantitated by statistical analysis of the differences between the two images. Also, the proportion or percentage of total tissue area stained may be readily calculated, as the area stained above an antibody threshold level in the second image.

Red and green filters are suitable for practice of the invention. For SA-β-Gal, a green filter (500 nm) is used to determine the total cytoplasmic area stained with red eosin and compared with the blue area stained for SA-β-Gal (600 nm) as determined using a red filter. This implementation shows a convenient and advantageous method for discriminating between two areas having counterstaining. It is recognized that there are various other staining or optical enhancement methods and filtering methods which can be used to optically enhance one particular area or feature over another cell feature (such as Fast green and eosin). The specific examples provided herein are suggestive of other equivalents for visualization of cell nuclei containing expressed p53, WAF1 protein, and SA-β-Gal.

Following visualization of nuclei containing p53 protein or WAF1 protein, the percentage of such cells in tissue derived from patients after treatment may be compared to the percentage of such cells in untreated tissue.

From these comparisons, the potential effectiveness of a putative therapeutic or chemopreventive agent against the cancer may be determined. Specifically, if there is a greater percentage of cells expressing p53, WAF1, SA-β-Gal, HSP60, or caspase 3 in the treated portion of the sample than there is in the untreated portion of the sample, then the therapeutic agent used in the treatment will be predicted to be potentially an effective agent for treating the cancer. Conversely, if there is a substantially equal percentage of cells expressing SA-β-Gal, p53, WAF1, HSP60, or caspase 3 in both the treated and untreated portions of the sample, or there is less protein expression in the treated portion of the sample, then the putative therapeutic agent will not be predicted to be effective for treatment of the cancer.

U.S. Patent Nos. 5,252,487 and 5,288,477, incorporated herein by reference, disclose one method of quantitating DNA and protein.

EXAMPLE 3

Response to Doxorubicin in Breast Cancer Cell Lines

Cellular changes are induced by treatment with moderate doses of doxorubicin and Taxol in breast tumor-derived human cell lines. MCF7 cells were treated for 2-3 days with 50 nM doxorubicin or Taxol (doses that induce growth inhibition and cellular death as measured by flow cytometry; as described by, *e.g.*, Dolbeare *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:5573-77) and then were stained for SA-β-Gal expression 10-11 days after release from the drug. The results of this treatment on the formation of sub-G_1 fraction and the SA-β-Gal$^+$ phenotype are shown in Figures 1A-1B and 2A-2C. Taxol-induced apoptosis is evident by the hypodiploid peak in the flow cytometry histogram, whereas doxorubicin treatment (and treatment with the monoclonal antibody Herceptin™) resulted in senescence and blocks at G_1, G_2, and G_2M as seen in SA-β-Gal$^+$ cells having the morphological features of senescence (Figures 2A-2C and Tables I and II).

EXAMPLE 4

Quantitation of SA-β-Gal Expression Following Treatment with Herceptin™

Frozen tumor sections were fixed and then stained for SA-β-Gal expression. SA-β-Gal$^+$ cells were quantitated using bright-field microscopy and image analysis. Tissues were obtained from a patient receiving only Herceptin™. The first needle biopsy was obtained from the metastatic disease before initiation of Herceptin™ therapy. On day 1 the patient received 4mg/lg.v Herceptin™ for 10 minutes. A second biopsy was taken on day 2, 24 hours after the patient received a second Herceptin™ treatment. A third biopsy was taken on day 14, 24 hours after the patient received a third dose of Herceptin™. The results of SA-β-Gal quantitation are shown in Figures 3A-3C and Table II.

EXAMPLE 5

Quantitation of p21^{WAF1} Expression Following Treatment with Doxorubicin

MCF7 cells were treated for 3 days with doxorubicin and then stained for p53 and p21$^{CIPI/WAF1}$ expression. Cells were cultured, stained, and analyzed as described above for SA-β-Gal using appropriate antibodies. The results of p53 and p21$^{CIPI/WAF1}$ quantitation are shown in Table III.

EXAMPLE 6

Quantitation of p21^{WAF1} Expression in Individuals Following Chemotherapy

Tissue samples from twenty breast cancer patients was analyzed for p21 expression by image analysis before and after neoadjuvant chemotherapy. Experiments were performed by the Imperial Cancer Research Fund (ICRF; Oxford University, U.K.). The results of this analysis show marked differences in tissue samples collected before and after treatment (Table IV).

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.